(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) Publication number: **0 197 390**
A2

(12) # EUROPEAN PATENT APPLICATION

(21) Application number: 86103842.0

(22) Date of filing: 26.04.84

(51) Int. Cl.⁴: C 07 D 209/16

(30) Priority: 01.07.83 US 510167

(43) Date of publication of application:
15.10.86 Bulletin 86/42

(84) Designated Contracting States:
AT BE CH DE FR IT LI LU NL SE

(60) Publication number of the earlier application
in accordance with Art. 76 EPC: 0 130 303

(71) Applicant: SOCIETE DES PRODUITS NESTLE S.A.
Case postale 353
CH-1800 Vevey(CH)

(72) Inventor: Berthelet, Raymond
Ch. Vers-chez-Cochard 11
CH-1807 Blonay(CH)

(72) Inventor: Hirsbrunner, Pierre
El Centero Ch. des Romains
CH-1801 Les Monts-de-Corsier(CH)

(54) **Preparation of N-acetyl serotonine and melatonine.**

(57) A process for the preparation of N-acetyl serotonine characterised in that serotonine is acetylated to form N, O-diacetyl serotonine which is then treated with an alkaline mixture of water and a lower alcohol to selectively hydrolyse the O-acetyl group of the N, O-diacetyl serotonine to give N-acetyl serotonine. The melatonine is prepared by methylating the N-acetyl serotonine in the 5-position.

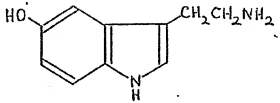
EP 0 197 390 A2

N-acetyl serotonine and melatonine 0197390

Preparation of ~~Serotonine and derivatives~~

The present invention relates ~~to a process for the separation of serotonine from coffee wax and also~~ to processes for the preparation of certain derivatives of serotonine, more particularly N-acetyl serotonine and melatonine. ~~and mexamine~~.

Serotonine, the chemical name of which is 5-hydroxytryptamine, is an indolic alkaloid having the following formula:



I

This alkaloid plays an important role in the metabolism of the brain and has vasoconstrictor, antihypertensive and antiallergenic properties, and may be used for the treatment of psychoses, migraine and for the control of excessive smoking.

The derivatives of serotonine also possess pharmacological properties as follows:

N-acetyl serotonine, the chemical name of which is N-acetyl-5-hydroxytryptamine has antihypertensive properties.

Melatonine the chemical name of which is N-acetyl-5-methoxytryptamine, is secreted by the pineal gland and possesses a regulatory activity on the circadian cycle. In addition, its use in an amount of 1-2 mg/day can induce ovulation in sheep, which is of considerable economic importance. Moreover, it has been shown that melatonine can induce sleep in man in an amount of 1-3 mg/kg body weight.

~~The serotonine may be isolated from the reaction medium~~ which contains it by conventional methods~~,~~ exploiting the fact that serotonine is a ~~e~~compound which has basic characteristics ~~and thus~~ has a minimum solubility in water at ~~pH about 10.8 (its isoelectric point).~~

The present invention ~~also~~ provides a process for the preparation of N-acetyl serotonine characterised in that serotonine is acetylated to form N, O-diacetyl serotonine which is then treated with an alkaline mixture of water and a lower alcohol to selectively hydrolyse the O-acetyl group of the N, O-diacetyl serotonine to give N-acetyl serotonine.

The serotonine used for the process may be obtained by any method, and may conveniently be prepared from coffee wax, preferably by the process of this invention. The acetylation may be carried out by conventional means, for instance, by the addition of excess acetylating agent, preferably acetic anhydride. The acetylation forms a mixture containing a major part of N, O-diacetyl serotonine together with a small amount of the desired N-acetyl serotonine. These two acetylated derivatives are advantageously extracted from the acetylation medium by means of a solvent substantially insoluble in water e.g. iso-butanol, preferably at pH 7, and then conveniently concentrating the organic phase to obtain an oil containing the two acetylated derivatives. This oil is then selectively hydrolysed, conveniently by dissolving in an alkaline mixture of water and alcohol in an amount from 2.5 to 7.5 times the volume of oil, preferably at a pH above 11 to produce the N-acetyl serotonine. The alcohol preferably has a boiling point below 100°C and conveniently contains from 1 to 4 carbon

atoms and is conveniently methanol, ethanol, n-propanol or isopropanol. The selective hydrolysis may conveniently be carried out a temperature from 15°C to 50°C, preferably from 25°C to 40°C, over a suitable period of time, for instance from 15 to 60 minutes. The pH may be adjusted by the addition of 30% sodium hydroxide solution.

The present invention further provides a process for the preparation of melatonine characterised in that N-acetyl serotinone is methylated in the 5-position. Any conventional methylating agent may be used, especially dimethyl sulphate which may, for instance, be added in excess to the aqueous-alcoholic solution of N-acetyl serotonine prepared as hereinbefore described. The pH is preferably above 11 and may be adjusted by adding a 30% sodium hydroxide solution while the temperature preferably does not exceed 45°C. During the reaction a considerable part of the melatonine crystallises and may be separated mechanically e.g. by filtration after which the reaction medium may be extracted, by conventional means, with a suitable solvent to recuperate the remainder of the melatonine. Examples of solvents that may be used are dichloromethane, chloroform, isobutanol and higher alcohols, ethyl acetate and some fluoro-chloro alkanes e.g. Freons, Halons.

~~The present invention also provides a process for the~~ production of mexamine characterised in that melatonine is deacetylated in a hot alkaline solution containing a substantially water-insoluble alcohol and then washed with water after which the alcohol phase is separated from the aqueous phase and acidified with hydrochloric acid.

Conveniently, the reaction mixture is cooled, for instance ~~to ambient temperature, before washing with water~~.

~~Example 1~~

Extraction of serotonine

700g of decaffeinated coffee wax containing 5% water were hydrolysed under an inert atmosphere of nitrogen after the addition of 300g ethyleneglycol monobutyl ether, 200g of potassium hydroxide and 12g of sodium dithionite. After 4 hours reaction at 140°C, the amides of serotonine were completely hydrolysed, and the mixture was cooled to 75°C, diluted with 1000g water and then acidified with 420g of 32% hydrochloric acid. The aqueous phase which formed was separated and the organic phase again extracted with 1200g of 0.1% hydrochloric acid at 75°C. After separation, the two aqueous phases were mixed, neutralised to pH 7 and filtered. 3000g of a solution containing 32g ~~of serotonine were obtained.~~

The following Examples further illustrate the present invention.

Example 2

Preparation of N-acetyl serotonine

To the aqueous solution of serotonine obtained in Example 1, there were added 40g acetic anhydride while maintaining the pH between 8-9 with 30% sodium hydroxide at 25°C to 30°C. N, O-diacetyl serotonine formed, having the appearance of an insoluble gum, and was extracted twice with 500g isobutanol. The extract thus obtained was concentrated to obtain 80g of an oil which was dissolved in a mixture containing 320 parts water and 80 parts ethanol. The pH was adjusted to 12.5 with 30% sodium hydroxide and the solution maintained at about 30°C for 30 minutes, which provokes the selective hydrolysis of the O-acetyl group. The solution then contained 36g

N-acetyl serotonine.

Example 2

Preparation of melatonine

To the aqueous alcoholic solution containing N-acetyl serotonine prepared in Example 2, there were added slowly and simultaneously 36g of dimethyl sulphate and 20g of 30% sodium hydroxide so that the pH was maintained at 12.5, while ensuring that the temperature did not exceed 40°C. During this operation, a part of the melatonine formed crystallised and this was filtered after neutralisation. The mother-liquor of crystallisation was decolourised with activated carbon, concentrated to eliminate the ethanol, then extracted with dichloromethane. After separation of the aqueous phase, the organic phase was concentrated to dryness which allowed the recuperation of a further 28g of crude melatonine. The two fractions, which totalled 42g, were mixed and purified by recrystallisation in a mixture containing water and ethanol in a 75:25 ratio. 30g of white crystals of melatonine were obtained having a purity of 98.6%.

~~Example 4~~

Preparation of mexamine

The melatonine obtained in Example 3 was taken up in 300g isobutanol. To this mixture were added 30g sodium hydroxide and 3g of sodium dithionite and the whole mixture was refluxed at 105°C for 2 hours under nitrogen. The

CLAIMS

1 1. A process for the preparation of N-acetyl serotonine characterised in that serotonine is acetylated to form N, O-diacetyl serotonine which is then treated with an alkaline mixture of water and a lower alcohol to selectively hydrolyse the O-acetyl group of the N, O-diacetyl serotonine to give N-acetyl serotonine.

2 2. A process according to claim 1 characterised in that the serotonine is prepared by the process according to claim 1.

3 3. A process according to claim 1 characterised in that the amount of water and alcohol used is from 2.5 to 7.5 times the volume of the N, O-diacetyl serotonine.

4 4. A process according to claim 1 characterised in that during the selective hydrolysis the pH is above 11.

5 5. A process according to claim 1 characterised in that the lower alcohol has a boiling point below 100°C and contains from 1 to 4 carbon atoms.

6 6. A process according to claim 1 characterised in that the selective hydrolysis is carried out at a temperature from 25°C to 40°C over a period of from 15 to 60 minutes.

7 7. A process for the preparation of melatonine characterised in that N-acetyl serotonine prepared by the process according to claim 7 is methylated in the 5-position.

8 8. A process according to claim 7 characterised in that excess dimethylsulphate is used as the methylating agent at a pH above 11 and at a temperature no higher than 45°C.